UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/518,749	12/22/2004	Takashi Nakayama	1422-0651PUS1	3018

2292 7590 10/27/2008

BIRCH STEWART KOLASCH & BIRCH
PO BOX 747
FALLS CHURCH, VA 22040-0747

EXAMINER
SGAGIAS, MAGDALENE K

ART UNIT	PAPER NUMBER
1632	

NOTIFICATION DATE	DELIVERY MODE
10/27/2008	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mailroom@bskb.com

Advisory Action Before the Filing of an Appeal Brief	**Application No.** 10/518,749	**Applicant(s)** NAKAYAMA ET AL.	
	Examiner MAGDALENE K. SGAGIAS	**Art Unit** 1632	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 02 October 2008 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☐ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:
   a) ☐ The period for reply expires _____months from the mailing date of the final rejection.
   b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

   Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☒ The Notice of Appeal was filed on *10/2/08*. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☐ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   (a) ☐ They raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☐ They raise the issue of new matter (see NOTE below);
   (c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: _____. (See 37 CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☐ For purposes of appeal, the proposed amendment(s): a) ☐ will not be entered, or b) ☐ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.
   The status of the claim(s) is (or will be) as follows:
   Claim(s) allowed: _____.
   Claim(s) objected to: _____.
   Claim(s) rejected: _____.
   Claim(s) withdrawn from consideration: _____.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
10. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See Continuation Sheet.
12. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____
13. ☐ Other: _____.

/Anne-Marie Falk/
Primary Examiner, Art Unit 1632

Continuation of 11. does NOT place the application in condition for allowance because:

A. Rejections under 35 USC § 102

Applicants respectfully submit that the Examiner appears to misunderstand Weiss. In Weiss, although the title at column 12, Example 3 recites "Isolation and Propagation of Embryonic Stem Cells", it is disclosed at lines 24-25 that "neurospheres" are actually formed. Thus, the title should have been "Isolation and Propagation of Neural Stem Cells". The embryonic stem cells used by Weiss are different from those presently claimed, for example, as shown in the attached Yu and Thompson, Genes & Development, 22:1987-1997 (2008). The embryonic stem cells in Weiss actually mean stem cells (i.e., neural stem cells) prepared from embryonic tissue (or embryonic brain). That is, Weiss uses "embryonic stem cells" as a common term to indicate neural stem cells from an embryo. This is apparently a misuse in comparison with the current definition. See also the following articles regarding the neurosphere method Reynolds et al., J. Neurosci. 12:4565-4574 (1992) and Rietze et al., Methods in Enzymology 419:3-23 (2006). As Applicants have previously argued, embryonic stem cells have an ability of differentiating into all of the cells - ectoderm, endoderm, or mesoderm. However, there has not been an instance where neural stem cells prepared from the brain are differentiated into germ cells. Accordingly, the presently claimed embryonic stem cells are quite different from the neural stem cells of Weiss. Thus, the present invention cannot be anticipated from Weiss.

These arguments are not persuasive because the method step of the instant claim 1 requires a suspension culture of embryonic stem cells in the presence of an astrocyte conditioned medium or ingredients equivalent to the conditioned medium to directly produce isolated neural cells. The instant claim does not require derivation of embryonic stem (ES) cells from a specific source as long as the ES cells are cultured in suspension and the astrocyte conditioned medium or the ingredient equivalent to the conditioned medium. Weiss teaches the source of ES cells is an embryonic day 14 (E14) CD1 albino mice brain and striata and the ES cells proliferated within the first 48 hours and by 3-4 days in vitro (DIV) they formed neurospheres that lifted off the substrate between 4-6 DIV (example 3). This source of ES taught by Weiss does not preclude containing ES cells as claimed in the instant invention. For example, the cited reference by the Applicants, Yu and Thompson, Genes & Development, 22:1987-1997 (2008) teach "The efficiency of mouse ES cell derivation is strongly influenced by genetic background, for example, ES cells can be easily derived from some non-permissive strains using modified protocols and mouse ES cells have also been derived from cleavage stage embryos and even from individual blastocysts of two to eight-cell stage embryos (p 1988, 1st column under Mouse ES cell heading). Furthermore, Yu and Thompson, teach medium that is "conditioned" by coculture with various cells was found to be able to sustain mouse ES cells via regulation of various signal transduction pathways (p 1988 1st column bridge to 2nd column). The Reynolds reference cited by Applicants teach a multipotent EGF-responsive striatal embryonic progenitor cell produces neurons and astrocytes and the Rietze reference teaches neural stem cell isolation and characterization. Neither of said cited references is relevant to the source of ES cells from an embryonic day 14 (E14) CD1 albino mouse brain and striata as in Weiss reference. Therefore, the instant rejection is maintained because Weiss teaches mouse ES cells in suspension in the presence of ingredient equivalent to produce neural cells as claimed in the instant invention. As discussed in the previous office action the invention as claimed requires the isolated neuron to express tyrosine hydroxylase and Weiss et al teach the isolated neuron expresses tyrosine hydroxylase as in the claimed invention. In addition, Weiss teaches the presence of ingredients equivalent to astrocyte conditioned medium as in the claimed invention where the cells are isolated in a suspension of embryonic stem cells in the presence of ingredients equivalent to an astrocyte conditioned medium in the state of adhesion of the neural stem cells to an adhesive culture substratum by plating the cells onto poly-L-ornithine coated glass cover slips, in the complete medium with rat B49 glial cell line-derived conditioned medium in the absence of bFGF, in the presence of FGF2 as in the claimed invention.

Therefore, the rejection is maintained.

B. Applicants argue Zhang reports that human embryonic stem (ES) cells cannot be maintained in an undifferentiated state in the absence of FGF-2 (Ref. 4 of Zhang). From this report, it is considered in Zhang that the differentiation occurs by removing FGF-2 and forming embryonic bodies (EB). Therefore, if FGF-2 is added, the differentiation would have been suppressed. These arguments are not persuasive because Ref 4 of Zhang by Amit is entitled "Clonally derived human embryonic stem cell lines maintain pluripotency and proliferating potential for prolonged periods of culture" but in the instant case the Zhang reference is not used for such type of rejection but for the use of ingredients equivalent to the conditioned medium and the absence of EGF as required in the instant claimed invention.

Applicants argue the neural stem sphere used in the present invention, the suppression of differentiation into the neural stem cells (NSC) does not take place even when FGF-2 is added upon suspension culture in ACM. Thus, the present invention possesses a synergistic effect, contrary to that described in Zhang, which enhances the differentiation. The effect for FGF-2 upon the suspension culture as described above is reversed; therefore, it is clear that the present invention is completely different from that taught in Zhang. The method of Zhang allows differentiation of the cells by an EB method to form neural tube-like structure on day 7 of the adhesion culture. By contrast, in the present invention, a sufficient amount of neural stem cells (NSC) are differentiated day 4 after the suspension, more in the suspension culture, not the adhesion culture. In the present invention, it is reasonable to consider that the reason that the NSC are differentiated only on a surface layer of the neural stem sphere is that a factor in ACM penetrates into the neural stem sphere, thereby enhancing the differentiation into NSC, proving that the ES cells are subjected to direct differentiation. The NSC are reportedly neuroepithelial stem cells and radial glia of the ventricular zone in embryo, or astrocytes of the subvenlricular zone and subgranular zone in adult (See Attachment 1: Doetseh, F., Nature NeurascL 6, 1127-1134, 2003). The fact that the NSC are differentiated in the order of neuroepithelial stem cells --> radial glia --> astrocytes, strongly suggests that the differentiation into astrocytes occurs in a default state of brain development. Therefore, the fact that almost all of the ES-derived NSC is differentiated into astrocytes by removing FGF-2 agrees with the phenomenon in the development of the brain in a living body.

These arguments are not persuasive because Zhang reference is used in the instant rejection for the claimed invention of claims 1-3 and 10-12 as cited in the office action pages 5-6 and specifically for is used for the limitations of ingredients equivalent to the conditioned medium and the absence of EGF as required in the instant claimed invention (see for example claim 10). The effect of FGF2 upon differentiation or time of differentiation or order of differentiation of stem cells is not requirement of the claimed invention.

Applicants argue upon the differentiation into the neural cells in the brain, firstly the differentiation into neurons occurs, and subsequently the differentiation into astrocytes and oligodendrocytes occurs in accordance with the progress of the time axis (See Attachment 2: Temple, S., Nature 414, 112-117, 2001). The ES-derived NSC of the present invention are differentiated into astrocytes in a default state as mentioned above, while almost all of the cells are differentiated into neurons by providing an exogenic differentiation stimulation called ACM. The NSC of Zhang is differentiated into three kinds of neural cells, namely neurons, astrocytes, and oligodendrites. Taking into consideration the axis of time from the development of the brain, it is understood that the neural stem cells of the present invention are more undifferentiated than those of Zhang, so that the cells of the present inventions seem to exhibit the nature close to that of the neuroepithelial stem cells. In order that small elongated cells congregated in the center shown in Fig. 1-A of Zhang form a neural tube-like structure shown in Fig. 1-B with the time course, Zhang merely mentions an experimental tool to study human neural tube formation under controlled conditions (Zhang, p. 1131, second column, second paragraph), i.e. ES cell-derived neural precursor cells, recapitulate early steps of nervous system development in that neural tube-like structures are formed, merely stating that the process of development is reproduced. Therefore, Zhang does not directly relate to the differentiation of the ES cells into NSC. Flat cells are migrated in the periphery of the adherent EBs; however, these cells are negative against markers for neurons, astrocytes, oligodendrites, and ES ceils. Therefore, under the conditions of Zhang, many of ES ceils are differentiated into unidentified cells; therefore, it is obvious that the ES cells cannot be directly differentiated into NSC. Art advantage of the Zhang method is to collect the cells only having a rosette structure utilizing the difference in adhesion from unidentified fiat cells, but never describing that Zhang performs direct differentiation of the ES cells.
Again these arguments are not persuasive because the instant invention does not require a temporal information for neural stem cell differentiation into neurons, astrocytes or oligodendrocytes as described in the reference of Temple. Zhang teaches human ES cells generate all three CNS cell types in vitro and expanded as neurospheres as required in the instant invention in a medium equivalent to the astrocyte conditioned medium. It is not required in the present invention a certain percentage of ES cells differentiating into NSC, or a certain region of the neurosphere (central or peripheral region of the neurosphere) having the NSC, therefore whether the percentage of differentiated cells in Zhang reference is different from that of the instant invention is irrelevant.

Applicants argue the article contribution of Flax describes NSC collected from human fetal telencephalon that is cryopreserved, so that Flax is distinguishable from the teachings of the present invention in the cell species.
These arguments are not persuasive because it would have been obvious for an ordinary of skill in the art to use conditions of Flax for the cryopreservation of NSC as for human fetal telencephalon of Flax.
Therefore, the instant rejection is maintained.

C. Rejections under 35 USC § 102/103

Applicants argue Pataky does not cure the deficiencies discussed with regard to Zhang. Pataky reports that neuronal axons of the CNS are regenerated in the spinal cord injuries (Refs. 65 and 66 of Pataky). While Pataky makes references to these publications, the main theme of the article is to evaluate what sort of factors enhance survival in regenerable bulbospinal neurons against injuries caused by axotomy. The effect of enhancing survival of ACM is such that astrocyte-conditioned medium also enhances the survival of bulbospinal neurons, supporting the hypothesis that non-neuronal cells are important mediators of trophic effects observed in vitro (page 366, second paragraph, last sentence of Pataky), to expect the enhancement of the survival by nonneuronal cells (including astrocytes) in the periphery of the injured spinal neurons. The bulbospinal neurons prepared from E8 Embryo retrograde-labeled with DiI have already ended differentiating into neurons (within the developing chick brain stem, neurogenesis is complete prior to E5; page 367, first paragraph, first sentence of Pataky), so that outgrowth of neurites from bulbospinal neurons is caused by regeneration. Therefore, Pataky which acknowledges that the differentiation into neurons is ended can no way expect the effect of nerve cell differentiation in ACM.
In response to applicant's argument that references 65 and 66 of Pataky the test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference; nor is it that the claimed invention must be expressly suggested in any one or all of the references. Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art. See In re Keller, 642 F.2d 413, 208 USPQ 871 (CCPA 1981). Zhang differs from the claimed invention by not teaching the culturing of stem cell spheres in the presence and then in the absence of bFGF and/or EGF for obtaining glial cell as a cell migrating from the stem cell sphere. Pataky teaches that fibroblast growth factor produced differential effects on survival and neurite outgrowth from identical bulbospinal neurons in vitro. Pataky teaches that astrocytes synthesize a variety of trophic factors and astrocyte conditioned medium also promoted the survival of bulbospinal neurons. Therefore, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the neural stem cell methodology of Zhang by progressive steps of adding bFGF or EGF to obtain neural stem cells and then culture the stem cells in the presence of bFGF or EGF to obtain glial cells with a reasonable expectation of success because Pataky states that astrocytes produce neural nutritional factors such as FGF2. Other teachings of Pataky with regard to ACM also enhances the survival of bulbospianl neurons supporting the hypothesis that non-neuronal cells are important mediators of trophic effects observed in vitro does not interfere with the use of Pataky reference for making up the deficiency of Zhang for culturing cells of stem cell spheres in the presence and then in the absence of bFGF and/or EGF for obtaining glial cells as a cell migrating from the stem cell sphere.
Therefore, the rejection is maintained..